#12/C
6/20/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 60953/119

In re patent application of:
HINTSCHE *et al.*

Serial No.: 09/142,660

Filed: December 23, 1998

For: *DETECTION OF MOLECULES AND MOLECULE COMPLEXES*

Group Art Unit: 1655

Examiner: Bradley L. Sisson

AMENDMENT AND REPLY UNDER 37 C.F.R. § 1.111

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

In reply to the Office Action dated December 8, 1999, please amend the application as set forth below and consider the following Remarks.

IN THE SPECIFICATION

At page 3, line 7, please insert:

- - This invention provides a method for the detection of molecules or molecule complexes in a diluent or solvent, wherein a sample to be measured is brought into contact with an ultra-microelectrode arrangement which has at least two electrode structures that are uncovered in the direction of the measurement area and arranged relative to one another such that the distances between the various structures lie in the ultra-micro range, wherein an alternating electric field is produced by application of an electric potential, and wherein the changes in current or potential, which are caused by species present or created in the

C1

continue C1

sample to be measured, is measured. - -

At page 3, lines 7-9, please delete "The way in which this object is achieved according to the invention is described in claim 1. The further claims present preferred refinements."

At page 4, line 18, before "They are," please insert:

- - In particular, the active electrode surfaces may consist of gold, platinum, C2 iridium or other noble metals, of carbon materials or of other conductive materials or of combinations thereof. - -

At page 4, line 18, please delete "(claim 16)".

At page 4, line 23, please delete "(claim 17)".

At page 5, line 3, please replace "a to d" with "Fig. 1a to 1d".

At page 5, line 10, please delete "(claim 19)".

At page 5, line 10, before "It is in this way," please insert:

- - Or the electrode structures may be arranged as multilayer structures that are C3 insulated from one another. - -

At page 5, line 20, after "lead on the chip," please insert:

- - and/or electronic components - -

At page 5, line 20, please delete "(claim 20)".

At page 6, line 25, please delete "(claims 3 and 4)".

At page 7, line 9, please delete "(claim 6)".

At page 7, line 14, please delete "(claim 7)".

At page 7, line 14, before "According to the," please insert:

- - In particular, amperometric oxidations, reductions, or redox recycling of C4 molecules having electrically active groups, or redox recycling of redox mediators may be induced and subsequently measured. - -

At page 8, line 6, before "In this case," please insert:

- - The molecules bound on the electrode surfaces by electropolymerization are C5 measured in the bound state. - -

At page 8, line 6, please delete "(claim 9)".

At page 8, line 20, please delete "(claim 10)".

At page 9, line 21, please delete "(claim 11)".

At page 10, line 5, please delete "(claim 12)".

At page 11, line 3, please delete "(claim 13)".

At page 11, line 12, please delete "(claim 14)".

IN THE FIGURES

In Figure 1, please replace "a," "b," "c," "d" and "e" with "1a," "1b," "1c," "1d" and "1e," respectively.

IN THE CLAIMS

Please cancel, without prejudice or disclaimer, claims 1-20.

Please add the following claims:

--21. A method of detecting a molecule or molecule complex in a diluent, solvent or gel, comprising:

(a) contacting the molecule or molecule complex with an ultra-microelectrode array, said ultra-microelectrode array comprising at least two electrode structures, wherein the spacing between the electrode structures is less than 3 μm;

(b) producing an alternating electric field between the electrode structures; and

(c) measuring changes in current or potential between the electrode structures, whereby the changes in current or potential are caused by the molecule or the molecule complex.

22. A method according to claim 21, wherein the electrode structures are applied to or incorporated in an insulating support material and are uncovered by an insulating support material in the direction of the measurement.

23. A method according to claim 21, wherein the changes in current or potential are measured using impedance spectroscopy.

33. A method according to claim 31, wherein the molecule or molecule complex binds to the surface of the electrode structures via self-assembling.

34. A method according to claim 31, wherein the molecule or molecule complex binds to the surface of the electrode structures via electropolymerization.

35. A method according to claim 21, wherein the molecule or molecule complex is positioned in the gap between the electrode structures.

36. A method according to claim 35, wherein the molecule or molecule complex is positioned by chemical binding, adhesion, or condensation reactions.

37. A method according to claim 21, wherein the ultra-microelectrode array comprises a molecular layer, the molecular layer comprising a second molecule that binds to the molecule or molecule complex to be detected, and whereby the binding between the second molecule and the molecule or molecule complex to be detected is capable of causing the changes in current or potential between the electrode structures.

38. A method according to claim 37, wherein the molecular layer contacts with a surface of the electrode structures.

39. A method according to claim 37, wherein the second molecule comprises biotin.

40. A method according to claim 37, wherein the second molecule comprises an antigen, and wherein the molecule or molecule complex to be detected comprises an antibody.

41. A method according to claim 37, wherein said second molecule comprises an antibody, and wherein the molecule or molecule complex to be detected comprises an antigen.

42. A method according to claim 37, wherein the second molecule comprises a first polynucleotide, and the molecule or molecule complex to be detected comprises a second polynucleotide capable of binding to the first polynucleotide.

43. A method according to claim 42, wherein the second polynucleotide binds to the first polynucleotide via hybridization.

44. A method according to claim 37, wherein the second molecule comprises a first and second polynucleotides, wherein the molecule or molecule complex to be detected comprises a third polynucleotide, and wherein the first, second and third polynucleotides are capable of forming a triple helix.

45. A method according to claim 21, wherein the ultra-microelectrode array comprises a first molecular layer and a second molecular layer, wherein the first molecular layer contacts the second molecular layer, wherein the second molecule layer comprises a second molecule, wherein the second molecule is capable of binding to the molecule or molecule complex to be detected, and whereby the binding between the second molecule and the molecule or molecule complex to be detected is capable of causing the changes in current or potential between the electrode structures.

46. A method according to claim 21, wherein a surface of the electrode structures comprises a layer of conductive material.

47. A method according to claim 46, wherein the layer of conductive material comprises a noble metal.

48. A method according to claim 46, wherein the layer of conductive material comprises carbon materials.

24. A method according to claim 21, wherein the changes in current or potential are measured independently of time, as a function of time or as a function of the phase angle.

25. A method according to claim 21, wherein the changes in current or potential are caused by diffusion or binding of the molecule or molecule complex to the ultra-microelectrode array.

26. A method according to claim 21, wherein the electrode structures are stacked, and comprise crossover points that are insulated from one another.

27. A method according to claim 21, wherein the alternating electric field comprises, is superimposed, or excited with a direct-current component.

28. A method according to claim 27, wherein the direct-current component induces an electrochemical reaction.

29. A method according to claim 28, wherein the electrochemical reaction comprises an oxidation or reduction of an electrically active molecule.

30. A method according to claim 28, wherein the electrochemical reaction comprises amperometric oxidations, amperometric reductions, redox recycling of a molecule having electrically active groups, or redox recycling of a redox mediator.

31. A method according to claim 21, wherein the molecule or molecule complex binds to a surface of the electrode structures.

32. A method according to claim 31, wherein the molecule or molecule complex binds to the surface of the electrode structures via physical or chemical binding.

49. A method according to claim 21, wherein the electrode structures are applied to or incorporated in an insulating material.

50. A method according to claim 49, wherein the insulating material is selected from the group consisting of silicon compounds, glass, ceramic and organic polymers.

51. A method according to claim 21, wherein the electrode structures are arranged to have a band structure, a strip structure, a circular structure or a finger-like interdigital structure.

52. A method according to claim 21, wherein the electrode structures are insulted from each other by an insulating material.

53. A method according to claim 52, wherein the insulating material is selected from the group consisting of silicon oxides, nitrides, ceramic and plastics.

54. A method according to claim 21, wherein the electrode structures are arranged to have a multi-layer structure with each layer insulated from one another.

55. A method according to claim 21, in which a direct or alternating current, or a combination thereof, is applied to at least one surface of the electrode structures, via insulated supply leads or electronic components, or a combination of thereof.

56. A method of detecting a molecule or molecule complex in a diluent, solvent or gel, comprising:

(a) contacting the molecule or molecule complex with an ultra-microelectrode array, said ultra-microelectrode array comprising at least two electrode structures, wherein the spacing between the electrode structures is less than 1 μm;

(b) producing an alternating electric field between the electrode structures; and

(c) measuring changes in current or potential between the electrode structures, whereby the changes in current or potential are caused by the molecule or the molecule complex.

57. A method of detecting a molecule or molecule complex in a diluent, solvent or gel, comprising:

(a) contacting the molecule or molecule complex with an ultra-microelectrode array, said ultra-microelectrode array comprising at least two electrode structures, wherein the spacing between the electrode structures is about the size of a large molecule complex;

(b) producing an alternating electric field between the electrode structures; and

(c) measuring changes in current or potential between the electrode structures, whereby the changes in current or potential are caused by the molecule or the molecule complex.

58. A method according to claim 21, wherein a second molecule binds to a surface of the electrode structures, wherein the molecule or molecule complex to be detected is capable of binding to the second molecule, and wherein the binding between the second molecule and the molecule or molecule complex to be detected dissociates the second molecule from the surface of the electrode structures.

59. A method according to claim 26, wherein the changes in current or potential are measured sequentially, in parallel or simultaneously.

60. A method according to claim 22, wherein the insulating support material is selected from the group consisting of silicon compounds, glass, ceramic and organic polymers. - -

REMARKS

Introduction

This communication replies to the Office Action mailed December 8, 1999. In the Action, the Examiner objects to the numbering of panels in Figure 1. The Examiner also objects to the reference to the claims in the specification. The Examiner rejects claims 1-20 under 35 U.S.C. 112 as being either indefinite or not enabling.

Applicants have amended the specification to delete the references to the claims. Applicants have also added several new sentences in the specification at line 7 on page 3, line 18 on page 4, line 10 on page 5, line 20 on page 5, line 14 on page 7, line 6 on page 8. These new sentences generally recite the language of the original claims 1, 16, 19, 20, 7 and 9, respectively. Therefore, the new sentences do not introduce new matter into the specification. Applicants have amended the numbering of the panels in Figure 1 to replace "a," "b," "c," "d" and "e" with "1a," "1b," "1c," "1d" and "1e," respectively.

Claims 1-20 have been canceled without prejudice or disclaimer. New claims 21-60 have been added. The subject matter of claims 21-60, in general, corresponds to that of claims 1-20. In particular, Applicants have replace the term "the ultra-micro range" in claim 1 with the terms "less than 3 μm," "less than 1 μm" and "about the size of a large molecule complex" in claims 21, 56 and 57, respectively. These terms are supported at page 3, lines 15-26, of the specification. Applicants have also replaced the term "active electrode surfaces" in claims 16 and 20 with the term "a surface of the electrode structures" in claim 46. Applicants have substituted the term "ultra-microelectrode array" for the term "ultra-microelectrode arrangement." The term "ultra-microelectrode array" is supported at page 3, line 13, of the specification. Applicants have replaced the term "fixed" in claim 10 with the term "positioned" in claim 35. The term "positioned" is supported at page 8, line 18, of the specification. Applicants have deleted the terms "reverse," "bound," "event" and "intersecting" in new claims.

Applicants believe that the amendments of the specification and claims do not introduce new matter into the application. Accordingly, Applicants respectively request allowance of the amendments.

Examiner's Rejections

Objection to the Specification

In paragraph 1 of the Action, the Examiner objects to the specification because of the existence of informalities. Applicants respectfully traverse the objection.

The Examiner objects to the numbering of the panels in Figure 1 as being informal. Applicants have amended the specification to replace the a, b, c, d, and e numbering with 1a, 1b, 1c, 1d and 1e numbering, respectively. Applicants respectfully submit that the amendment obviates the Examiner's rejection.

The Examiner also objects to the references to the claims in the specification. Applicants have deleted all the references to the claims in the specification. Accordingly, Applicants respectfully submit that the Examiner's objection is rendered moot.

Rejection of Claims 1-20 Under 35 U.S.C. § 112, First Paragraph

In paragraph 3 of the Action, the Examiner rejects claims 1-20 under 35 U.S.C. § 112, first paragraph, as not enabling one of skill in the art to make or use the invention. Applicants have canceled claims 1-20 and added new claims 21-60. The subject matter of claims 21-60, in general, corresponds to that of claims 1-20. Applicants respectfully traverse the rejection.

The Examiner contends that, "while being enabling for coating of an electrode with SH-biotin and detection/measurement of B-galactosidase and p-aminophenol, [the specification] does not reasonably provide enablement for the detection of any chemical in any type of sample." Applicants respectfully direct the Examiner to claim 21 of the present application. Claim 21 prescribes a method for detection of a molecule which is capable of causing "the changes in current or potential between the electrode structures." Accordingly, the present invention does not provide a method "for the detection of any chemical in any type of sample," as contended by the Examiner. Rather, the present invention detects only those molecules that are capable of causing detectable current or potential changes. In addition, the present invention detects the existence, as opposed to the nature, of "a" molecule that is capable of causing current or potential changes. In other words, if molecule A and B co-exists in a solution and both molecules are capable of producing current or potential changes, the present invention might not distinguish them under certain circumstances. However, the present invention can determine that at least

one molecule exists in the solution because of the changes in current or potential. Detection of current or potential changes has been well known to a person skilled in the art. Accordingly, Applicants respectfully submit that the present invention satisfies the enablement requirements and therefore the Examiner's rejection is rendered moot.

In paragraph 3 of the Action, the Examiner also contends that the present invention may not be used for detection of molecules in a biological sample. Specially, the Examiner states that "the use of heterogeneous solutions present a plethora of difficulties, e.g., just which compounds is one measuring/detecting . . .." As discussed above, the base claim 21 prescribes a method for detection of the existence, as opposed to the nature, of "a" molecule or molecule complex which can cause current or potential changes. The identity of the molecule might not be determined by the claimed method. Rather, the existence of "a" molecule in a biological sample can be determined if the molecule can cause current or potential changes. Therefore, Applicants submit that the present invention can be applied to biological or other heterogeneous samples as long as these samples comprise at least "a" molecule that can produce a detectable current or potential change.

Applicants note that under certain circumstances, the present invention may be used for determination of the nature of the molecule. For example, an antigen-coated electrode may be used for detection of the existence of an antibody in a biological sample, wherein the antibody can specifically bind to the coated antigen and such binding produces a detectable change in current or potential. In this example, other components in the biological sample might also cause current or potential changes. However, an ordinarily skilled in the art would know that these background current or potential changes can be eliminated by using routine control experiments. For instance, a control sample may be made by adding free antigens to the original sample. The added free antigens may saturate the antibody and therefore prevent the antibody from binding to the antigen coated on the electrodes. The difference between the current or potential changes caused by the control sample and that caused by the original sample may be used to determine the existence as well as the nature of the antibody.

In paragraph 3 of the Action, the Examiner further contends that "[t]he specification does not set forth a repeatable procedure whereby one would be able to measure any compound . . .." As discussed above, the present invention prescribes methods for

detection of the existence of a molecule which can cause current or potential changes. Measurement of current or potential changes is well known to a person skilled in the art. Accordingly, Applicants respectfully submit that the specification provides sufficient guidance and therefore enables one of skill in the art to measure the current or potential changes, including those produced by the molecule or molecule complex as prescribed in claim 21.

The Examiner contends that the specification is silent as to how to measure hybridization of nucleic acids to complementary sequences. Applicants respectfully direct the Examiner to claim 42, which is dependent from claim 37. Claim 42 prescribes a method for detection of the existence of a polynucleotide which can bind to another polynucleotide that is coated on the surface of the electrode structures, wherein the binding between the two polynucleotides can cause current or potential changes. Consequently, the present invention detects the current or potential changes, rather than the specific ways in which the two polynucleotides bind to each other. Accordingly, Applicants believe that long as the binding creates a detectable change in current or potential, the present application provides sufficient guidance to enable a person skilled in the art to detect such binding.

Applicants note that the present invention may be used to determine the strength of binding between the two polynucleotides, for example by changing the salt concentration in the sample. The strength of binding may subsequently be used to infer the extent of hybridization between the two polynucleotides. However, the claimed invention is not restricted to detection of hybridization. Rather, any interaction that causes current or potential changes may be detected by the claimed invention. As noted above, measurement of current or potential changes is known to the art. Therefore, Applicants respectfully submit that the present invention meets the enablement requirements under 35 U.S.C. § 112, first paragraph.

Rejection of Claims 1-20 Under 35 U.S.C. § 112, Second Paragraph

In paragraphs 5-12 of the Action, the Examiner rejects claims 1-20 under 35 U.S.C. § 112, second paragraph, for failing to particularly point out and distinctly claim the subject matter of the invention. Applicants respectfully traverse the rejection.

In paragraphs 5 and 10 of the Action, the Examiner rejects claim 1. The Examiner finds that the term "the ultra-micro range" lacks sufficient antecedent basis and therefore is indefinite. Applicants have canceled claim 1. In the corresponding new claim 21, Applicants replace the term "the ultra-micro range" with the term "less than 3 μm." The term "3 μm" is supported by the specification at page 3, line 25. Similarly, , Applicants use the term "less than 1 μm" instead of "the ultra-micro range" in claim 56. The term "1 μm" is supported by the specification at page 3, line 26. In claim 57, Applicants use the term "about the size of a large molecule complex." This term is described by the specification at page 3, lines 15-16. Applicants believe that an ordinary skilled in the art would understand this term, particularly in view of various routine technologies available for determination of the size of a molecule complex. Based upon the foregoing, Applicants respectfully submit that the new claims obviate the Examiner's rejection..

In paragraphs 6 and 8 of the Action, the Examiner rejects claims 16 and 20, respectively. The Examiner contends that the term "the active electrode surfaces" lacks sufficient antecedent basis. Applicants have canceled claims 16 and 20. In new claims 46 and 55, Applicants use the term "a surface of the electrode structures" or the term "at least one surface of the electrode structures" instead of "active electrode surfaces." Accordingly, Applicants respectfully submit that the new claim overcomes the Examiner's rejection.

In paragraph 7 of the Action, the Examiner rejects claim 18 for reciting the term "the micrometer." The Examiner alleges that the term "micrometer" has insufficient antecedent basis. Applicants respectfully direct the Examiner to page 3, line 25, of the specification, where it states that the minimum spacing of the electrodes themselves should "typically be less than 3 μm, preferably 1 μm." Applicants believe that the term "micrometer" is a term of art and that an ordinarily skill in the art would understand that the term "μm" denotes the term "micrometer." Accordingly, Applicants respectfully submit that the term "micrometer" is definite and therefore the Examiner's rejection is rendered moot.

In paragraph 9 of the Action, the Examiner rejects claim 1 for reciting the allegedly indefinite term "ultra-microelectrode arrangement." Applicants have replaced the term "ultra-microelectrode arrangement" with the term "ultra-microelectrode array." The term

"ultra-microelectrode array" is defined at page 3, lines 12-16, of the specification., where it states that an "ultra-microelectrode array" has "electrode structures . . . arranged so closely next to one another that they approach the size of large molecule complexes, for example immunoprotein or DNA molecules." The specification further defines, at page 3, lines 24-26, that a "ultra-microelectrode array" has its electrodes arranged with a minimum spacing of "typically less than 3 μm, preferably 1 μm." Based upon the foregoing, Applicants believe that the term "ultra-microelectrode array" is sufficiently defined in the specification and therefore is definite. Accordingly, Applicants respectfully submit that the Examiner's rejection is obviated.

In paragraph 11 of the Action, the Examiner rejects claim 7 as being nonsensical. Applicants have canceled claim 7. The corresponding new claim 30 reads "A method according to claim 28, wherein the electrochemical reaction is produced by amperometric oxidations, amperometric reductions, redox recycling of a molecule having electrically active groups, or redox recycling of a redox mediator." Applicants respectfully submit that the new claim is readable and therefore overcomes the Examiner's rejection.

In paragraph 11 of the Action, the Examiner rejects claim 8 for using the allegedly indefinite term "bound." Applicants have canceled claim 8. The corresponding claim 32 reads "A method according to claim 31, wherein the molecule or molecule complex binds to the surface of the electrode structures via physical or chemical binding." The terms "binds" and "binding" are described in the specification at page 7, lines 20-26, and therefore are definite. Accordingly, Applicants respectfully submit that the new claim obviates the Examiner's rejection.

In paragraph 11 of the Action, the Examiner rejects claim 10 for reciting the allegedly indefinite term "fixed." Applicants have canceled claim 10. In the corresponding new claim 35, Applicants substitute the term "positioned" for the term "fixed." The specification defines the term "position" at page 8, lines 16-28. Accordingly, Applicants respectfully submit that the term "positioned" is definite and therefore overcomes the Examiner's rejection.

In paragraph 11 of the Action, the Examiner rejects claim 11 for reciting the allegedly indefinite term "reverse." Applicants have canceled claim 11. Applicants do not

use the term "reverse" in any new claims. Accordingly, Applicants respectfully submit that the new claims obviate the Examiner's rejection.

In paragraph 11 of the Action, the Examiner rejects claims 13 and 14 for being indefinite with respect to the term "event." Applicants have canceled claims 13 and 14. Applicants do not use the term "event" in any new claims. Accordingly, Applicants respectfully submit that the Examiner's rejection is rendered moot.

In paragraph 12 of the Action, the Examiner rejects claims 13 and 14 as being incomplete for omitting essential steps. In particular, the Examiner contends that the following steps are omitted: (1) how the electrode surface is coated with a second molecule; (2) how the hybridization reaction is performed; (3) how one can differentiate between multitudes of different nucleic acids in the sample or between differing degree of complementarity. Applicants have canceled claims 13 and 14. The corresponding new claims are claims 42 and 44, respectively.

The Examiner appears to allege that the essential step for coating a molecule, particular a polynucleotide, to an electrode surface is omitted in claims 42 and 44. However, Applicants believe that the technique for coating molecules, including polynucleotides, to an electrode has been well known to the art. Particularly, the specification describes at page 11, lines 4-5 that polynucleotides can be coated to a electrode surface via thiol bonds. Accordingly, Applicants believe that one of skill in the art would know how to coat a polynucleotide molecule to the electrode surface. Therefore, Applicant respectfully submit that there is no essential step omitted from claims 40 and 42.

In addition, the methods prescribed in claim 42 and 44 allow detection of the interactions between polynucleotide molecules, provided that the interactions can produce detectable changes in current or potential. The present invention detects only changes in current or potential, not the nature of the interactions. These interactions may or may not involve hybridization. Accordingly, Applicants submit that the present invention does not prescribe direct methods for determination of "how" the hybridization is performed or to what degree complementarity is involved. Therefore, Applicants respectfully submit that the Examiner's rejection is rendered moot.

In paragraph 19 of the Action, the Examiner rejects claim 19 for being "confusing as to how the electrodes are insulated from one another yet can be intersecting with one

another." Applicants have canceled claim 19. The corresponding new claim 54 does not recite the term "intersecting." Accordingly, Applicants respectfully submit that the new claim obviates the Examiner's rejection.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully submit that the present claims are in condition for allowance. An early notice in this regard is respectfully requested. Should the Examiner have any questions regarding the present application or believe that further discussion will advance prosecution, the Examiner is invited to contact the undersigned at the number listed below.

Respectfully submitted,

June 8, 2000

Date

Matthew R. Cohen
Reg. No. 40,960

Foley & Lardner
3000 K Street, NW
Washington Harbour, Suite 500
Washington, DC 20007
Tel: (202) 672-5300
Fax: (202) 672-5399

Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees.